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Resumo

A competição interespecífica é um processo que ocorre quando uma ou mais espécies partilham os mesmos recursos limitantes. Este processo afeta, normalmente, de forma diferente as espécies envolvidas, dependendo do suas capacidades competitivas e adaptativas. Frequentemente, pode levar a alterações nas dinâmicas das populações e das comunidades das espécies envolvidas.

As espécies invasoras, beneficiadas pela falta de inimigos naturais fora da sua distribuição original, representam uma elevada pressão competitiva para as espécies nativas das áreas invadidas que partilhem os mesmos recursos, podendo levar a alterações na abundância e riqueza específica nas comunidades envolvidas. Assim, é relevante estudar os efeitos da competição entre espécies invasoras e residentes.

O Ácaro-aranha vermelho, *Tetranychus evansi* Baker and Pritchard, de origem Sul-Americana é uma espécie invasora de sucesso, tendo estendido a sua distribuição por diversos países africanos e chegado à Europa via países mediterrânicos. É uma espécie especialista em plantas Solanáceas, incluindo espécies utilizadas em práticas agrícolas (Tomate, Batata, Beringela, Tabaco, etc). Não tendo predadores naturais fora da sua área de distribuição nativa e tendo mostrado bastante tolerância à maioria dos acaricidas, tem-se tornado uma praga nos ecossistemas que invadiu, competindo com espécies do mesmo género e dominando áreas onde anteriormente espécies nativas, em particular *Tetranychus urticae* Kosh, eram dominantes, nomeadamente na Bacia Mediterrânica. Apesar disso, existem diversos registos de coexistência entre T. evansi e *T. urticae*, tendo sido as duas espécies encontradas inclusivamente nas mesmas plantas. Por outro lado, em estudos laboratoriais, foi também observada em determinadas condições, a superioridade competitiva de *T. evansi* relativamente a *T. urticae,* em plantas de tomate. *T. evansi* provoca da diminuição de resposta defensiva da planta de tomate, sendo ambas as espécies favorecidas por este processo. No entanto, *T. evansi* beneficia da produção de uma teia densa, excluindo assim o competidor. Estes dados indicam que o resultado da competição interespecífica

entre estas duas espécies pode ser bastante variável. Desta forma, torna-se bastante pertinente o estudo das interações entre as duas espécies, de forma a melhor compreender a ecologia desta espécie invasora, permitindo definir as estratégias de controlo biológico para o futuro.

Assim, o presente trabalho tem como objetivo estudar a interação entre a espécie invasora, *T. evansi*, e a residente, *T. urticae*. Em particular, pretende-se determinar se o padrão de colonização de plantas numa das espécies é afetado pela presença da espécie competidora.

Primeiro, verificou-se para ambas as espécies se existia uma preferência por folhas mais jovens (4ª folha) ou mais antigas (2ª folha) quando colonizando uma nova planta de tomate não infestada. Para tal foram testados vários métodos em que se conectava uma arena, onde posteriormente os ácaros eram libertados (N = 100), com a 2ª e a 4ª folha de uma planta de tomate com 27 ou 28 dias. Isolaram-se ambas as folhas com lanolina à volta do pecíolo, próximo do caule, para que os ácaros não pudessem dispersar para outras folhas, nem aceder às folhas testadas de outra forma que não diretamente da arena. Os métodos utilizados foram paus de madeira e diversos tipos de fios. Não tendo sido encontradas diferenças significativas no número de ácaros recapturados nas plantas depois de 24h através dos vários métodos, as restantes experiências foram realizadas utilizando fios de nylon. O número de ácaros em cada uma das folhas foi contado em intervalos de 1h30m durante 6h e novamente 24h após a libertação dos mesmos na arena.

Verificou-se, para ambas as espécies uma preferência por folhas mais jovens (4ª folha), provavelmente por ainda estarem em crescimento e terem portanto uma menor capacidade de defesa. Possivelmente também por serem folhas cuja posição relativa é mais elevada e portanto proporcionando futuramente uma melhor dispersão aérea.

Investigou-se de seguida se a presença da espécie competidora numa planta de tomate, influenciava a preferência verificada anteriormente para ambas

as espécies. Com este intuito, desenvolveu-se uma experiência em que se introduziam 20 ácaros de uma das espécies na planta 48 horas antes de libertar os 100 ácaros da outra espécie. Outros detalhes experimentais são idênticos à experiencia anterior Plantas não infestadas foram preparadas de forma idêntica e utilizadas como controlo. Três tratamentos foram efetuados: no primeiro uma das espécies era introduzida previamente na 4ª folha e depois a outra era libertada; no segundo uma das espécies era introduzida previamente na 2ª folha e depois a outra era libertada; e finalmente, no terceiro tratamento, uma das espécies era introduzida previamente em ambas as folhas e depois a outra era libertada.

Verificou-se que a espécie *T. urticae*, provavelmente como consequência da competição interespecífica, evita o seu competidor movendo-se na planta para folhas não infestadas por *T. evansi*. Por outro lado, a espécie *T. evansi* altera a sua preferência para a 2ª folha, quando a competidora está presente, independentemente da posição desta última na planta.

Finalmente, com o objetivo de testar se as espécies em causa não só evitariam um competidor alterando a posição relativa na mesma planta, mas também movendo-se para uma planta vizinha, foram feitas experiências utilizando duas plantas, em que uma delas foi previamente infestada pelo competidor e a outra foi mantida limpa. Neste caso, foram conectadas as folhas de ambas as plantas, a uma arena, onde os 100 ácaros da outra espécie foram libertados. Foram efetuados 2 tratamentos em que foram apenas testadas folhas da mesma idade, eliminando os efeitos da qualidade da folha e da posição relativa. No primeiro caso, conectaram-se apenas as 4as folhas de ambas plantas à arena onde os ácaros foram libertados e no segundo caso conectaram-se apenas as 2asfolhas das duas plantas. Procedeu-se também a um último tratamento em que ambas as folhas de ambas as plantas foram conectadas à arena.

Num cenário com duas plantas, uma limpa e uma previamente infestada com a espécie competidora, o evitamento de *T. evansi*, pela espécie *T. urticae* não se verificou, não tendo esta mostrado preferência por plantas limpas comparativamente com plantas previamente infestadas por *T. evansi*. Uma

possível explicação para estas diferenças poderá estar relacionada com o facto da espécie *T. urticae*, quando em situação de co-infestação com *T. evansi*, poder beneficiar por esta causar a diminuição das defesas anti-herbívoria da planta de tomate, tornando-a mais suscetível ao ataque de herbívoros. Este benefício poderá compensar os custos da competição interespecífica. Por outro lado, a espécie *T. evansi*, neste sistema de duas plantas, mostrou ser atraída pela presença da espécie competidora preferindo a planta pré-infestada. Acresce-se que, no entanto, esta atração pela espécie competidora não foi sempre confirmada à escala de uma planta, visto *T. evansi* ter alterado a sua preferência para folhas mais antigas quando a competidora já se encontrava anteriormente na planta, independentemente da posição que esta última ocupava.

As diferenças de comportamento das duas espécies, na presença de uma espécie competidora, poderão estar ligadas com a distinta reação que estas provocam na planta de tomate. Por um lado *T. urticae* ativa as defesas anti-herbívoria da planta, tornando-a menos adequada ao estabelecimento de ambas as espécies. Por outro lado, *T. evansi*, diminui a resposta defensiva da planta, tornando-a mais suscetível à herbívoria. Acrescenta-se que estas diferenças na resposta defensiva da planta, causadas pelas duas espécies, podem ser propagadas sistemicamente de forma variada, consoante o tipo, a intensidade e o local em que o dano é causado pelo herbívoro, podendo os efeitos ser ou não sentidos noutras partes da planta. Assim sendo, a ordem de chegada destas espécies à planta e o local onde estas se estabelecem parece ser determinante relativamente ao efeito que uma espécie poderá ter na outra através da planta.

Os comportamentos de evitamento ou atração das espécies estudadas, uma em relação à outra, parecem ser importantes interações decorrentes da competição entre elas. Complementados com novos estudos relativos ao desempenho de ambas as espécies, na presença ou ausência da espécie competidora, em diferentes locais da planta, os resultados deste estudo podem ajudar-nos a compreender as consequências da competição interespecífica entre espécies invasoras e residentes.

Summary

Inter specific competition is a process that occurs between two or more species that share the same limiting resources. This process is normally asymmetric, affecting differently the implicated species, depending on the species adaptive and competitive abilities and frequently causes changes in the dynamics of the populations and communities of the involved species. Invasive species, commonly lacking natural enemies in the invaded areas, present a high competitive pressure to the native species that share the same resources, leading sometimes to changes in the abundance and richness of those. *Tetranychus evansi* Baker and Pritchard is an invasive species native from South America, which has invaded Europe through Mediterranean countries less than 20 years ago. It is a specialist in Solanaceae plants, having tomato as preferred host. This species competes with native species of the same genus and, in the Mediterranean basin has been found dominating areas where the native species were previously abundant. However, there are many reports of coexistence of *T. urticae* and *T. evansi* in the field, in Iberian Peninsula, suggesting inter-specific competition between these two species. In addition, in laboratory studies, *T.* evansi was able to out-compete *T. urticae* when using tomato as a host plant. Thus, it has become very pertinent to study the interactions between these two spider mite species. In this context, the present study aims to determine if the colonization pattern of these species is affected by the presence of the competitor species. We verified that both *T. evansi* and *T. urticae* have a preference for young leaves, when colonizing a new tomato plant. This behavior seems to be affected when the competitor species is already infesting the colonized plant. Depending on the relative position on the plant and the order of arrival, these species are differently influenced by their competitor. On one hand, *T. urticae* always avoided its competitor, moving within the plant and even losing its preference for the younger leaf when *T. evansi* was already pre-infesting it. On the other hand, *T. evansi* changed its preference to the older leaf, independently of the relative position of its competitor. Finally, preference for a clean versus an infested plant was tested comparing the colonization of two plants, one clean and the other pre-infested by

the competitor, both given as a choice to the tested spider-mites. For *T. urticae* there was no preference between clean and pre-infested plants but a preference for the younger leaf was confirmed. For *T. evansi*, preferred pre-infested plants and in the tandem of two plants, lost preference for the younger leaf. The results from this study point to an effect of the presence of a competitor in the colonization behavior of the target spider mites species. Nonetheless, this effect is variable in different situations and according to the species. Both species seem to profit from the presence of the other but relative position of both species in the plant and the order in which they arrive to plant seems to be relevant to this benefice.

Keywords: Invasive species; inter-specific competition; colonization; *Tetranychus evansi.*

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Introduction

Inter specific competition

Inter specific competition is a process that occurs between two or more species that share the same limiting resource. It affects adversely the species involved, either indirectly, by diminishing the common resource's availability, in case of exploitative competition, or directly - the case of interference competition - where some species interact negatively with the others (Krebs 1994, Begon et al. 1996). This process is usually asymmetric, affecting differently the involved species, depending on the species adaptive and competitive abilities and frequently causes changes in the dynamics of the populations and communities (Strong 1984, Kaplan and Denno 2007). Indeed, the species that can maintain the highest growth rate at a lowest resource level can lead to the exclusion or displacement of the competing species (Tilman 1982, Reitz and Tumble 2002). As a consequence, species can avoid their competitors through niche differentiation - specializing in different resources or exploiting the same resources at different times or different places (Brown and Wilson 1956, Krebs 1994). A particular form of this is the investment of one species on colonization skills. In such case, the least competitive species evolves the ability to colonize empty niches at a higher rate. These competition / colonization trade-offs allow different species to coexist in a metacommunity (Amarasekare 2002, 2003).

Additionally, avoidance can occur within a plant, as some herbivores move vertically on a plant, even to less edible old leaves, to escape from a predator, by detecting its presence with predator-related odors (Magalhães et al. 2002, Onzo et al. 2003), they should be as likely to avoid a competitor in the same conditions.

Invasive species and competition

When arriving to a new ecosystem, an invasive species will interact with native species, such as enemies, competitors or hosts. These interactions can result in changes in the structure and dynamics of the existing communities. On their new habitat, these invasive species are often free of their natural enemies, which keep

their populations in check in their original range. As a consequence, these species often represent a high inter specific competitive pressure on resident species which share the same limiting resources (Strauss et al. 2006).This pressure can lead to several outcomes, such as competitive exclusion of the invader or the resident, or character displacement Therefore, studying the competitive interactions between invasive and resident species will contribute to our understanding of the composition of ecological communities.

This seems to be happening with spider mites in the Mediterranean basin, since the introduction of *Tetranychus evansi* Baker and Pritchard. Many locations in Spain, where resident spider mite species, *Tetranychus urticae* Kosh and *Tetranychus turkestani* Ugarov and Nikolskii, were previously abundant, are now dominated by *T. evansi* (Ferragut et al 2007,2013). Nonetheless, there are many reports of coexistence of *T. urticae* and *T. evansi* in the field, in Iberian Peninsula, sharing the same plantations and even being found within the same plants. These findings support the possibility of inter specific competition between these two species (Escudero and Ferragut 1998, Ferragut et al. 2007). However, the outcome of these interactions is not yet very clear and may depend on several biotic and abiotic factors.

Distribution and invasive process of *Tetranychus evansi*

Spider mites are haplodiploid phytophagous species from the Acari family Tetranychidae. Most of the species are polyphagous. They occur in several agricultural and ornamental plants, inflicting severe damage on them and are thus considered important agricultural pests (Zhang 2003). Due to their small size, tolerance for extreme low and high temperatures and humidity and in some cases its ability to suffice without food for long periods of time, are among the most successful invasive organisms. The increasingly global movements of humans, animals, plants and resources in the latest centuries led to high levels of dispersion of these species. Being closely associated with plants, its relevance in economical

and medical is considerable, making its control a matter of great importance (Navajas and Ochoa 2013).

Tetranychus evansi is a spider mite species that has expanded from its natural distribution in South America, having been accidentally introduced in various locations and reported presently in several parts of the world, in all continents (Boubou et al. 2012). Although this species isn't currently considered a pest in its native range, where it's found only in a few locations (Furtado et al. 2007), it has become problematic in many countries in Africa, and, recently, in the Mediterranean basin. Its presence was first recorded in Zimbabwe in 1979 (Saunyama and Knapp 2003) but, due to its characteristics that allow its rapid growth under dry and hot conditions, it expanded to other African countries, reaching Southern Europe through the Mediterranean Sea and possibly through alternative introductions in the Iberian Peninsula (Boubou et al. 2012) , being presently found in Portugal, Spain, France, Italy and Greece (Bolland and Vala 2000,Ferragut and Escudero 1999, Migeon 2005, Castagnoli et al. 2006, Tsagkarakou et al. 2007).

This successful invasive species is specialized in plants from Solanacea family, colonizing a variety of plants used in agricultural practices, being Tomato, *Solanum lycopersicum*, its preferred host (Moraes et al 1987, Bolland et al. 1998). Not having natural enemies outside its native distribution (Furtado et al. 2006, Vasconcelos et al. 2008), and becoming resistant to many kinds of pesticides (Ferrero et al. 2011,Fiaboe et al. 2006), it has rapidly increased its distribution and numbers, becoming a high competitive pressure to the resident species of the same genus.

Thus, it becomes essential, not only in the context of Conservation Biology but also in a practical socio-economic context of agricultural practices, the study of this invasive herbivore's ecology and the relationships it establish with native host plants, competitor herbivores and predators, in order to understand the potential risks of this introduction in the Mediterranean region (Navajas et al. 2013).

The main species that *T. evansi* is expected to compete with in Europe is *T. urticae*, an abundant generalist spider mite, which occurs often in Solanaceous

plants (Gould 1979). Therefore, it is quite pertinent to study the interactions among these species, to generate predictions on the future composition of solanaceous crop pests in Europe.

The possible mechanisms underlying the success of *T. evansi*, using tomato as a host, were also researched. It was verified that, unlike *T. urticae*, that induces the production of anti-herbivory compounds by tomato plants, *T. evansi* provokes a reduction in host plant defenses, profiting from this condition and leading to an augmentation of its capacity to use this host (Sarmento et al. 2011a). However, this effect is also be profitable to other herbivores that would infest the plant. But, in addition, it was also verified that *T. evansi*'s intense web production has a negative effect on the competing species, protecting the plants from other herbivores and preventing them from benefiting from the defense down-regulation (Sarmento et al. 2011b).

Another comparison of the posture size of *T. urticae* and *T. evansi*, in the presence or absence of the competitor species was made, and different results were found depending on host plant. On one hand, *T. evansi*, being a specialist in plants of Solanacae family, increased its posture on tomato, when the competitor species, *T. urticae*, was present. The same didn't happen to *T. urticae,* when *T. evansi* was present. On the other hand, *T. urticae* almost doubled its posture size on bean, when *T. evansi* was present (Lozano 2010). Nonetheless, these experiments were performed introducing the mites at the same time in arenas made from already detached leaves. In this situation, plant defense was not up / down regulated by the mites, and consequently there was no effects on mite performance. *T. urticae*'s lower performance when its competitor was present, confirms the effect of *T. evansi*'s web on *T. urticae*.

Despite the fact that tomato is a preferential host for *T. evansi*, and that *T. urticae* seems to be out-competed by its competitor in this host, both species can be found in the field coexisting in tomato plantations. Besides, these two species seem to have different mechanisms to cope with tomato plant defenses, making it appealing to use it as the chosen host in this study.

Objectives of the present work

The present work, being an integrative part of a bigger project aiming to study the interactions established between a resident spider mite, *T. urticae* and an invasive spider mite, *T. evansi*, focuses on the possible interferences that one species can have in the competitor colonization process and vice versa.

The following questions were addressed:

1- When colonizing a new clean tomato plant, do *T. urticae* and *T. evansi* have a preference for and older leaf or a younger less defended one?

2- Will they modify their colonization behavior (losing or changing their preference) when the competitor is already present in the plant?

3- Will these species avoid their competitor within the same plant or into neighboring plants?

Material and Methods

Mites and plants

Tomato plants (Castlemart variety) were sowed in a greenhouse, in a 150 mm diameter pot, where they grew for 4 weeks (25 ºC, l:d = 16:8). Some of the 27-28 days old plants were used in the experiments and the remaining were used for mite rearing. Kidney bean plants (*Phaseolus vulgaris*), also sowed in a greenhouse with the same conditions (25 ºC, l:d = 16:8) , were also used for mite rearing.

T. evansi spider mites, previously collected in tomato plants sowed in greenhouse in Brazil in 2002, were reared on trays with tomato plants leaves on top of a humid cotton layer, using water as a barrier. New leaves were added every two days.

T. urticae spider mites, R5 strain, were collected in *Lamium spp*, a plant from Lamiaceae family, in the Netherlands in 2010, were reared on bean plants using a similar set-up as that for T. evansi.

All experiments were performed with mated female spider mites, two days post last quiescence stage. In this way, not only the sex and age factors were eliminated from the experiments, but also, the probability of having more mites establishing on a new a plant was increased. To ensure female mites used in the experiments had the same age, every week females where isolated on separate leaves, and allowed to lay eggs for 48h, being removed afterwards. The female offspring ecloding from these eggs had thus approximately the same age. Those individuals were used in the experiments.

The cultures of both species and developing mites to be used in the experiments were kept in climate rooms (25 Cº, 65% humidity, l:d = 16:8).

Within-plant distribution on clean plants

Preference of spider mites for an older (2^{nd}) or a younger (4^{th}) leaf was tested using a set up that consisted on a 35 mm diameter petry dish, connected directly to the 2^{nd} and the 4^{th} leaf of the plants. Different methods (wooden sticks or several types of strings) were used. In all cases, both leaves were isolated with lanolin to prevent the mites from dispersing to other parts of the plant. Afterwards, 100 mites, from one species or the other, previously collected and starved for at least one hour, were released in the arena. The number of mites on each leaf was recorded every 1h30 for 6 hours and 24h hours after the release. Plants with unconnected leaves to the arena, with stem climbing as the only way for the mites to reach the leaves, were used as control.

Within-plant distribution on plants with competitors

To test if the presence of a competitor species affected the distribution of spider mites, mites from the competing species (N = 20) were introduced on one leaflet of each plant with lanolin around the petiole, to prevent the mites from dispersing through the plant (hereafter "infested plants"). 48h later, 100 individuals of the tested species was released, in an arena connected directly to both leaves (2^{nd} and 4^{th}) with nylon strings. The number of mites from the latter species on each leaf was recorded every 1h30 for 6h and 24h after the release. As the main focus of these experiments was to investigate the effects of heterospecific competition on the colonization process of the target spider mite species, plants pre-infested with the same species were not used.

Three different treatments were prepared: i) 20 mites introduced on the 4^{th} leaf; ii) 20 mites introduced on the 2^{nd} leaf; iii) 20 mites introduced on each leaf. Clean plants arranged with the same set up, with also 100 mites being released at the same time as the different treatments, were used as control.

Choice between infested and clean plants

To test if the presence of a competitor affected mite choice between plants, mites were given the choice between clean plants and plants infested with competitors. The mite releasing arena was placed in a platform between the two pots. In each test, only the target leaves were connected directly to the arena with a nylon string. As before, three different treatments were tested: i) pre-infested 4^{th} leaf vs. clean 4^{th} leaf; ii) pre-infested 2^{nd} leaf vs. clean 2^{nd} leaf; iii) both leaves pre-infested vs. both leaves of a clean plant. As before, 20 mites from the competing species were introduced in the target leaves (2^{nd}, 4^{th} or both) 48h before releasing the tested species (N = 100) in the arena. The number of mites of the latter species on each leaf was recorded every 1h30 for 6h and 24h after release.

Statistical Analysis

All statistical analyses were performed with the software package R 2.15.2 (2012). The total number of mites recaptured on the plant after 24h was compared between methods using a Generalized Linear Model with Poisson distribution, or Quasi-Poisson, when the data was over dispersed. Replicates, methods and interactions between the previous, were used as fixed factors. The total number of mites recaptured on the plant after 24h was compared between treatments using a Generalized Linear Model with Poisson distribution, or Quasi-Poisson, when the data was over dispersed. Replicates, treatments and interactions between the previous, were used as fixed factors. Comparisons between the distribution of mites through the different leaves within the same treatment were performed using a Generalized Linear Model with Poisson distribution, or Quasi-Poisson, when the data was over dispersed. Leaf, replicates and the interactions between the previous, were used as fixed factors. Comparisons between the distribution of mites through the different leaves among different treatments were performed using a Generalized Linear Model with Binomial distribution, or Quasi-Binomial, when the data was over dispersed, using the proportion of mites that were on the 2^{nd} leaf after 24 hours, within the total number of mites on the plant after 24 hours

(2^{nd} leaf + 4^{th} leaf). Treatment, replicate and the interactions between the previous, were used as fixed factors. In all cases the model was simplified to the minimum adequate model by removing non significant (P>0.05) parameters or interactions.

Results

Within-plant distribution on clean plants

No significant difference was found in the average number of mites present on plants using different release methods, for both species ($F_{1:12}$ = 1.90, P = 0.19 for *T. urticae* and $F_{1:12}$ = 0.05, P= 0.84 for *T. evansi*, respectively). The number of *T. urticae* mites on the plants after 24 hours was significantly lower than that of *T. evansi* mites ($F_{1:26}$ = 10.72, P = 0.003) (Figure 1).

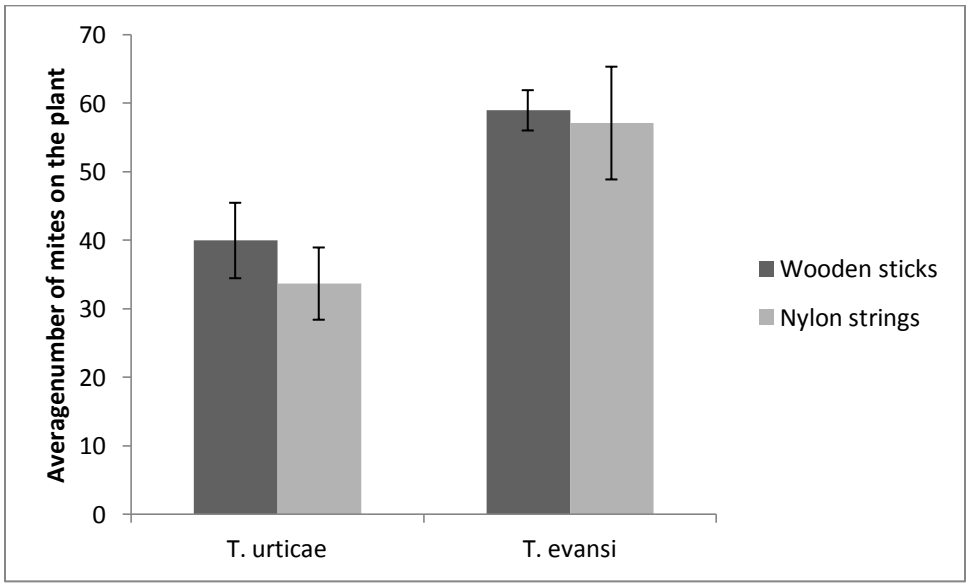

Figure 1. Comparison of methods connecting the releasing arena with the target leaves. Average number of mites on the plant (sum of the number of mites on the 2nd leaf and the number of mites on the 4th leaf) after 24 hours, per method, per species. Vertical bars correspond to standard error.

As there were no significant differences between methods, data in the subsequent tests was grouped.

T. urticae showed a preference for the 4th leaf ($F_{1:32}$= 72.99, P = $9.19e^{-10}$), with 81% of the total number of mites recaptured on the plant being present in the 4th leaf. *T. evansi* also showed a preference for the younger leaf ($F_{1:26}$= 64.62, P =

1.62e^{-08}), with 83% of the total number of mites recaptured on the plant being present in the 4th leaf (Figure 2).

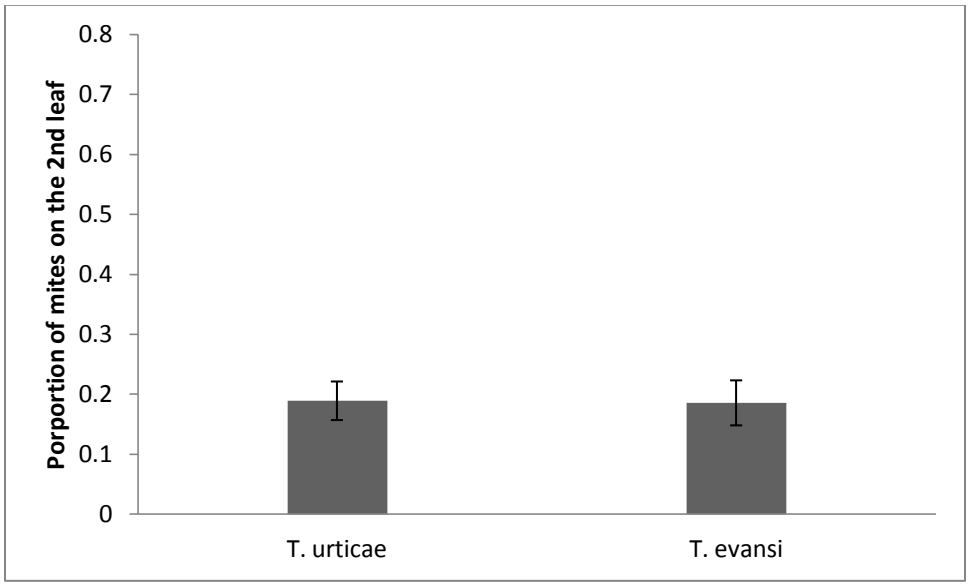

Figure 2. Mite preference between older and younger leaves. Average proportion of mites on the 2nd leaf of clean plants after 24 hours, for the two species. Vertical bars correspond to standard error.

Within-plant distribution on plants with competitors

Presence of a competitor species on the 4th leaf

We found no significant differences in the total number of mites recaptured on clean or infested plants, both for *T. urticae* ($F_{1:14}$= 2.28, P = 0.15), and for *T. evansi* ($F_{1:14}$= 0.95, P = 0.35) (Figure 3).

The proportion of *T. urticae* recaptured on the 2nd leaf after 24 hours on plants infested with *T. evansi* for 48 hours on the 4th leaf was significantly different from that on clean plants($F_{1:14}$= 43.56, P = 1.19^{e-05}). For *T. evansi*, the proportion of mites observed on the 2nd leaf after 24 hours on the treatment where the plants were pre-infested with *T. urticae* was also significantly different from the control($F_{1:14}$= 75.83, P = 5.03^{e-07}) (Figure 4).

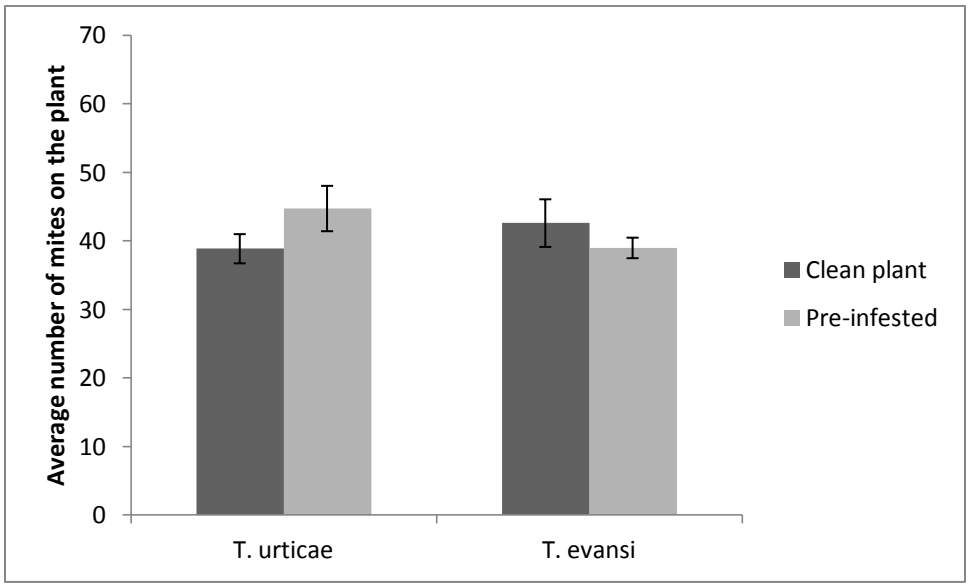

Figure 3. Comparison of the total number of mites recaptured after 24 hours. Average number of mites on the plant (sum of the number of mites on the 2nd leaf and the number of mites on the 4th leaf) after 24 hours, per treatment: clean plant and plant pre-infested with the competitor species on the 4th leaf; per species. Vertical bars correspond to standard error.

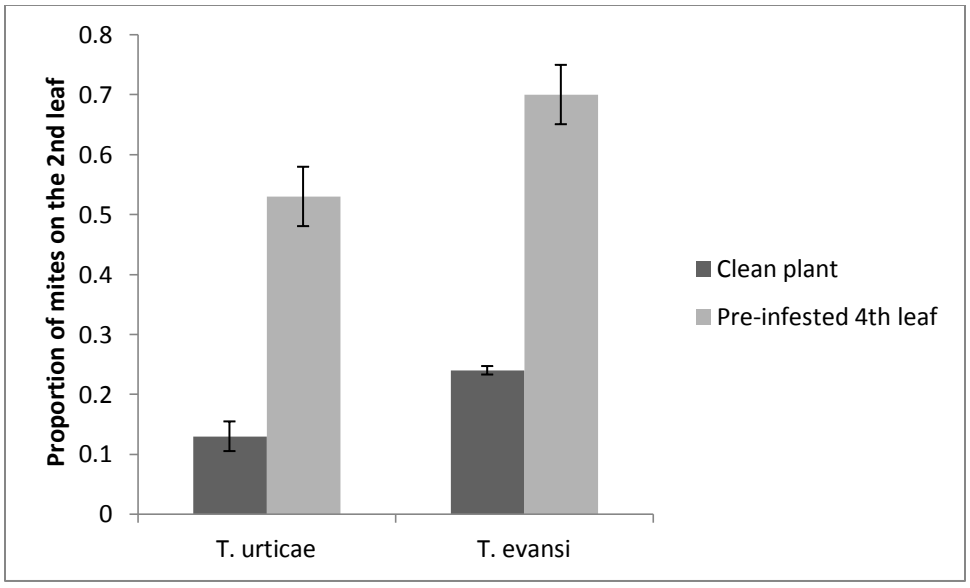

Figure 4. Mite preference between older and younger leave, with its competitor previously established on the youngest. Average proportion of mites on the 2nd leaf after 24 hours. per treatment: clean plant and plant pre-infested with the competitor species on the 4th leaf; per species. Vertical bars correspond to standard error.

Presence of a competitor species on the 2nd leaf

We found no significant differences in the total number of mites that were recaptured on clean and infested plants, for *T. evansi* ($X^2_{1:14}$= 7.43, P = 0.28). For *T. urticae*, the number of mites recaptured on the plant 24 hours after release on infested plants was significantly higher than on clean plants ($F_{1:14}$= 7.81, P = 0.01) (Figure 5).

The proportion of *T. urticae* recaptured on the 2nd leaf after 24 hours when the plants were pre-infested with *T. evansi* for 48 hours on the 2nd leaf was not significantly different from that on clean plants ($X^2_{1:14}$= 20.26, P = 0.40). In contrast, for *T. evansi*, the proportion of mites recaptured on the 2nd leaf after 24 hours on plants infested with *T. urticae* on the 2nd leaf, was significantly higher than on clean plants ($F_{1:14}$= 37.22, P = 2.74^{e-05}) (Figure 6).

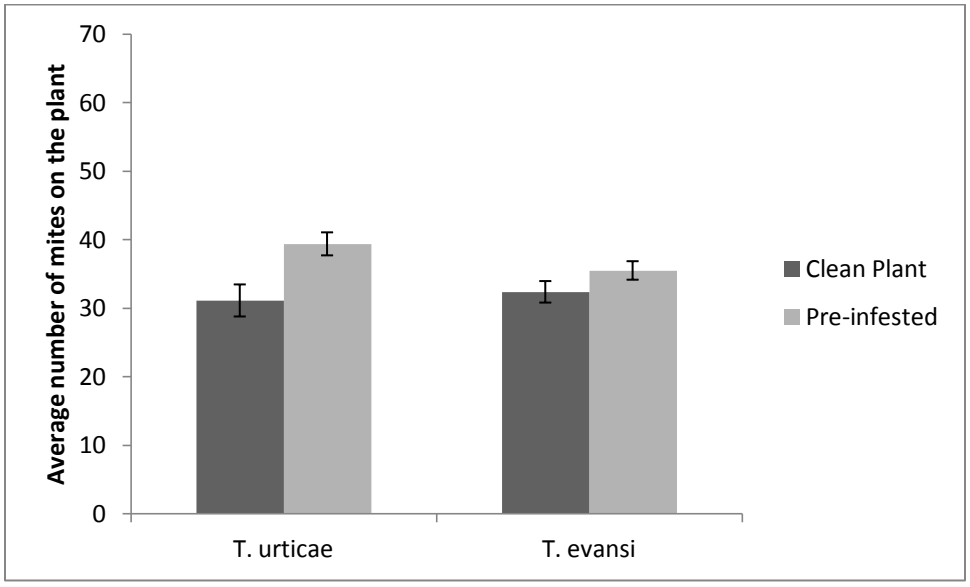

Figure 5. Comparison of the total number of mites recaptured after 24 hours. Average number of mites on the plant (sum of the number of mites on the 2nd leaf and the number of mites on the 4th leaf) after 24 hours, per treatment: clean plant and plant pre-infested with the competitor species on the 2nd leaf; per species. Vertical bars correspond to standard error.

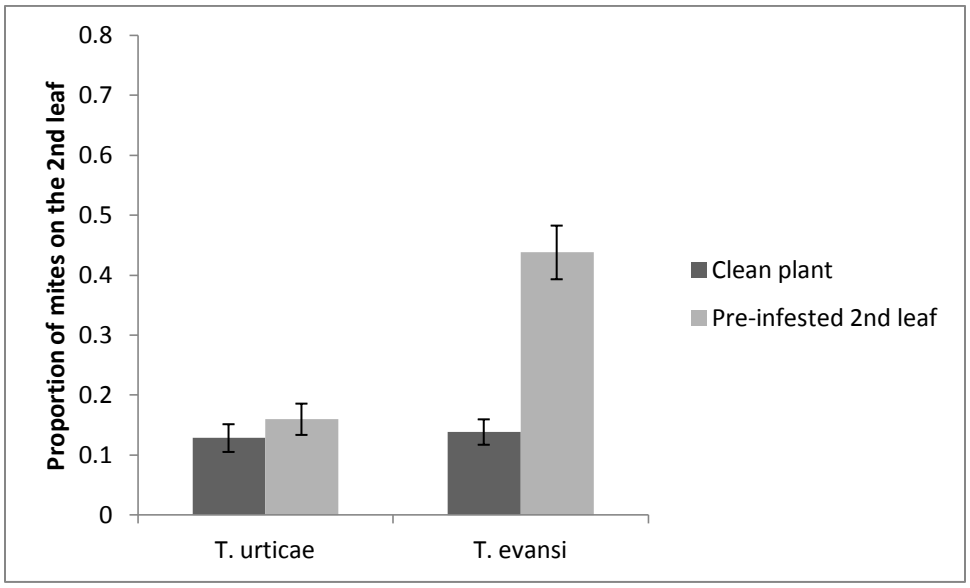

Figure 6. Mite preference between older and younger leave, with its competitor previously established on the oldest. Average proportion of mites on the 2nd leaf after 24 hours. per treatment: clean plant and plant pre-infested with the competitor species on the 2nd leaf; per species. Vertical bars correspond to standard error.

Presence of a competitor species on both leaves

We found no significant differences in the total number of mites that were recaptured on the plant 24 hours after release between clean and infested plants, both for *T. urticae* ($X^2_{1:13}$= 13.75, P = 0.56), and for *T. evansi* ($X^2_{1:14}$= 7.35, P = 0.053) (Figure 7).

The proportion of *T. urticae* recounted on the 2nd leaf after 24 hours when the plants were pre-infested with *T. evansi* for 48 hours on both leaves wasn't significantly different from control ($F_{1:13}$= 0.32, P = 0.58). In contrast, for *T. evansi*, the proportion of mites recounted on the 2nd leaf after 24 hours on the equivalent treatment, where the plants were pre-infested with *T. urticae* on both leaves, was significantly different from control ($F_{1:14}$= 104.47, P = 7.12^{e-08}) (Figure 8).

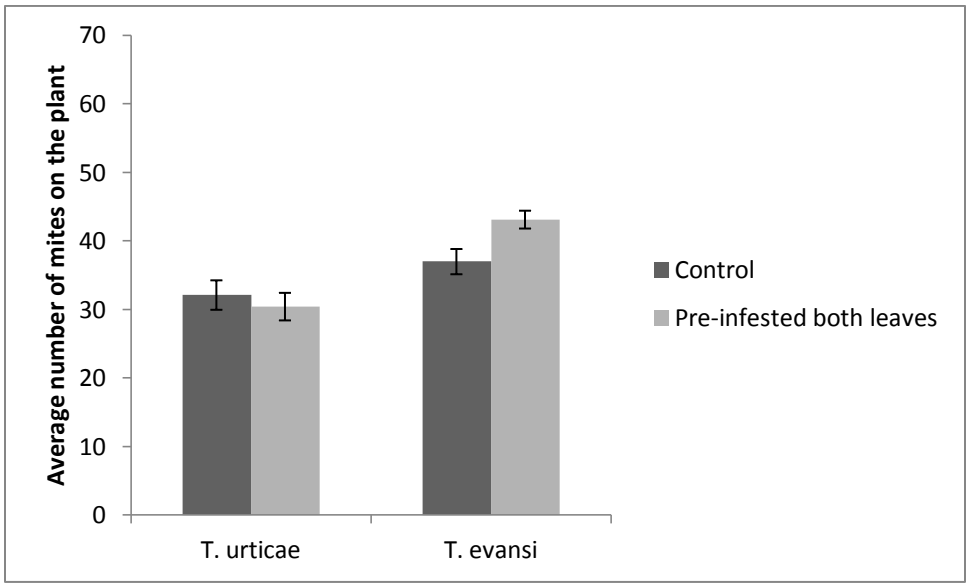

Figure 7. Comparison of the total number of mites recaptured after 24 hours. Average number of mites on the plant (sum of the number of mites on the 2nd leaf and the number of mites on the 4th leaf) after 24 hours, per treatment: clean plant and plant pre-infested with the competitor species on both leaves; per species. Vertical bars correspond to standard error.

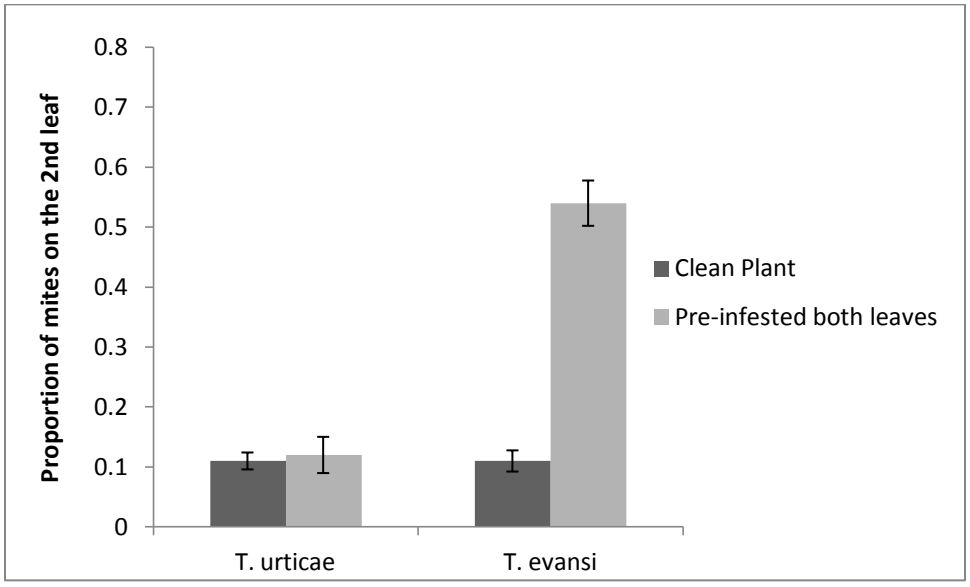

Figure 8. Mite preference between older and younger leaves, with its competitor previously established on both leaves. Average proportion of mites on the 2nd leaf after 24 hours. per treatment: clean plant and plant pre-infested with the competitor species on both leaves; per species. Vertical bars correspond to standard error.

Choice between infested and clean plants

Clean 4th leaf vs. Pre-infested 4th leaf

The number of mites on the 4th leaf after 24 hours, was not significantly different between pre-infested plant and clean plants, both for *T. urticae* ($F_{1:10}$= 1.65, P = 0.23) and *T. evansi* ($F_{1:10}$= 0.70, P = 0.42) (Figure 9).

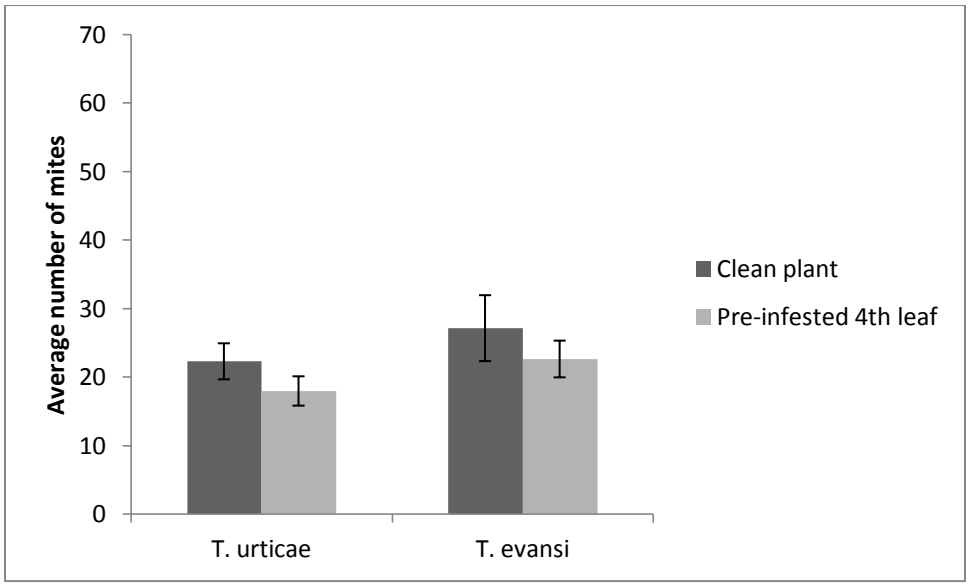

Figure 9. Mite preference between the 4th leaf of a clean plant or a plant pre-infested with its competitor. Average number of mites, for the two species, on the 4th leaf of each plant after 24 hours. Vertical bars correspond to standard error.

Clean 2nd leaf vs. Pre-infested 2nd leaf

For *T.* urticae, the number of mites on the pre-infested 2nd leaf after 24 hours was significantly lower than the number of mites on the 2nd leaf of the clean plant ($X^2_{1:12}$= 3.59, P = 0.0038). For *T. evansi*, the number of mites on the pre-infested 2nd leaf after 24 hours was significantly higher than the number of mites on the 2nd leaf of the clean plant ($F_{1:10}$= 42.8, P = $6.54e^{-05}$) (Figure 10).

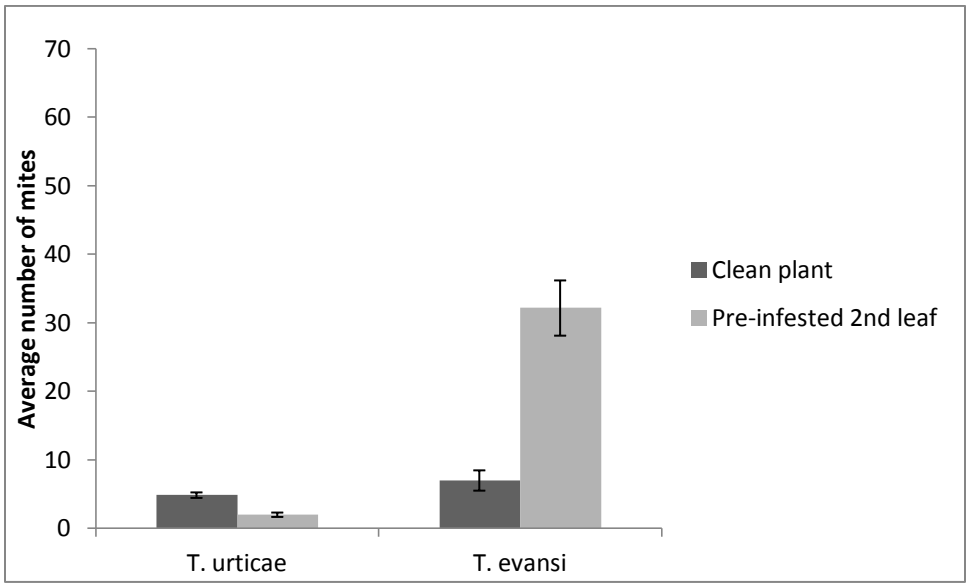

Figure 10. Mite preference between the 2nd leaf of: a clean plant or a plant pre-infested with its competitor. Average number of mites, for the two species, on the 2nd leaf of each plant after 24 hours. Vertical bars correspond to standard error.

Clean plants vs. plants with both leaves pre-infested

For *T. urticae*, the total number of mites after 24 hours on the clean plants was not significantly different from the number of mites on the pre-infested plants ($X^2_{1:10}$= 5.91, P = 0.26). For *T. evansi*, the total number of mites after 24 hours on the clean plants was significantly lower than the number of mites on the pre-infested plants ($F_{1:16}$= 31.61, P = $3.82e^{-05}$) (Figure 11).

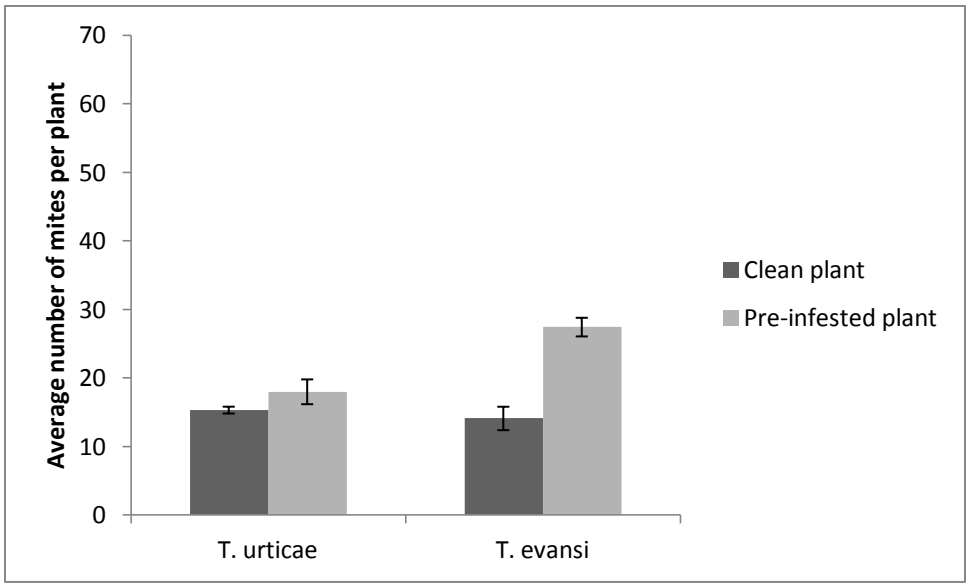

Figure 11. Comparison of the total number of mites recaptured on a clean plant and on a plant pre-infested with its competitor. Average number of mites on the plant (sum of the number of mites on the second leaf and the number of mites on the forth leaf) after 24 hours, per species. Vertical bars correspond to standard error.

Discussion

The results from our experiment show that both spider mite species, *T. urticae* and *T. evansi*, when colonizing a clean tomato plant, have a preference for younger leaves. However, both species behavior seems to be affected by the presence of their competitor on the plant, although they seem to be affected differently. *T. urticae* avoids its competitor within the same plant but does not discriminate between clean plants and plants infested with its competitor. In contrast, *T. evansi* changes its preference to an older leaf, when its competitor is already present in the plant, independently of the position of the latest. When given the choice between clean plants and plants pre-infested with its competitor, *T. evansi* preferred the infested plants.

Preference for younger leaves, as already seen for *T. urticae* in other plants species (Opit et al 2003, Kumaran 2011), may be due to the fact that younger leaves that are still growing are less defended than older fully grown leaves. This happens since the incoming resources are allocated to growth and not to defensive traits (Herms and Mattson 1992). The younger leaf is also the highest in the plant and spider mites could also choose it to establish as it is a better place for future dispersion (Kennedy and Smitley 1985). Such preference for younger leaves, in *T. urticae* and *T. evansi*, suggests that both species would preferentially establish in the same position within the plant. This is likely to intensify the inter-specific competition between these two species.

Avoidance of *T. evansi*, within the same plant, seems to be a possible response of *T. urticae* to inter-specific competition. Indeed, by avoidaing leaves with competitors, *T. urticae* can establish on empty leaves, and hence minimize the impact of competition, at least during the early stages of colonization. Such within-plant avoidance has been shown in another spider mites, the Cassava Green Mite, when avoiding predators present on different plant leaves (Magalhães et al. 2002). These small scale avoidance behaviors are highly relevant in a tri-trophic context because they are beneficial for the herbivore, but not for the plant. In contrast, between-plant avoidance is beneficial for both parties. Nonetheless, the presence

of *T. evansi*, at a broader scale, does not modify the colonization patterns of *T. urticae*, as this species shows no preference between clean plants and plants pre-infested by *T. evansi*. One possible explanation for this is that *T. urticae* when co-infecting the same plant as *T. evansi*, can profit from plant defenses down regulation caused by the latest (Sarmento et al. 2011b) and this may overcome the cost of inter-specific competition, as the shared resources are more suitable for both species. However, the effect of down regulation is local, but may not have an even systemic distribution, and *T. urticae* avoids leaves with *T. evansi* within a plant, hence it is not clear if this possibility holds. Another possible explanation is that *T. urticae* is unable to detect volatile cues associated with the presence of its competitor on plants.

This last justification does not seem to be in accordance with previous studies regarding various complex interactions among different trophic levels, including several involving *T. urticae*, which have shown that the presence of herbivores, due not only to plant defense volatiles but also by herbivore pheromones, is noticed by predators (Dicke 1986, Venzon et al. 1999), conspecific mites and other competitor herbivores (Pallini et al. 1997, Kant et al. 2004, Janssen et al 1997) and also neighboring plants (Baldwin 2002). Other studies have demonstrated differences of attractiveness of infested leaves and non-infested leaves of infested plants (Frost et al. 2007, Takabayashi et al 1991, Horiuchi et al 2003). Moreover, we have observed that, spider mites move up and down the string connecting both plants, so they are likely to detect the presence of the competitor. Still, we cannot rule out the possibility that, by down-regulating plant defences, *T. evansi* also limits the production of plant volatiles, making them less conspicuous to predators and competitors (Sarmento et al. 2011a).

The explanations previously proposed for *T.* urticae between plant behavior cannot also be used to explain *T. evansi*'s behavior, since *T. evansi* prefers pre-infested plants, compared to clean plants. However, this attraction for the competitor is contingent upon the leaf occupied by T. urticae, as *T. evansi*, when *T.*

urticae was already pre-infesting a plant, preferred the oldest leaf, regardless of the latest position on the plant.

These differences between the behavior of the two species may be related with the distinct mechanisms to cope with tomato plant defenses presented by the two species. On one hand, *T. urticae* triggers the defense response of the tomato plant, making it less edible and less suitable for both species. On the other hand, *T. evansi* down-regulates it, making it more suitable for both species (Sarmento et al. 2001b). In addition to these differences in plant response caused by these two species, the way plant anti-herbivory defense systemic system operates varies in intensity and distribution, with the type and position of damage. Depending on the part of the plant where it is provoked, reaction of the plant to damage might be felt differently through the plant (Stout 1996). Therefore, the order in which the species arrive to the plant and the position where they are established may be relevant and related to the effect it is going to have, or not, on the competitor, because the effects from up / down regulation of plant defenses mentioned above may be felt or not, in different parts of the plant.

Finally, the number of mites previously infesting the plant may also be relevant in the amount of damage caused and thus influence the intensity of the plant's defensive reaction. In these experiments, although plants were damaged after being infested with 20 mites for 48 hours, this damage could have been insufficient to cause up / down regulation of plant defenses, and thus, the effects on species could have in the other, trough the plant may not have been triggered. Presently, density studies are being performed to better understand its role on *T. evansi* and *T.urticae* competition behavior.

Nonetheless, none of the studies mentioned above regarding volatile cues were focused on within-plant avoidance or attraction. As our results demonstrate, these within-plant behaviors seem to be relevant regarding inter-specific competition between these two species. However, in order to better understand, how these species affect one another according to the position they occupy in the plant, performance of this two species, regarding different scenarios of co-

infestation, between different leaves, is being studied. Results from these studies, may corroborate these avoidance and attraction behaviors as a result of inter-specific competition. These new studies will also to confirm the connection of these behaviors to possible effects one species can cause to the other, though the plant.

Final remarks

The results from this study point to an effect of inter-specific competition in the colonization behavior of the target spider mites species. Nonetheless, this effect is dependent on the species, to the scale (within plant or between plants) and to the order of introduction.

Furthermore, within plant avoidance and attraction behaviors seem to have an important role in these species competitions interactions. Complemented by new studies, already being performed, regarding performance of both species in the presence or absence of the other, in different parts of the plant, these results can help us understand some consequences of inter-specific competition between this invasive spider mite, *T. evansi*, and other con-generic native species, such as *T. urticae.* Also, larger scale experiments would be needed to validate these results and enable extrapolations to field situations.

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